L Number	Hits	Search Text	DB	Time stamp
1	36104	|435/68.1|435/69.1|435/69.6|435/69.7|435/7[illegible]	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 14:32
2	8	"apical membrane antigen" and |435/68.1|435/69.1|435/69.6|435/69.7|435/7[illegible]	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 14:32
-	2	6207371.pn.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 14:26
-	32	"apical membrane antigen"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 14:32
-	5768	plasmodium	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:35
-	238561	kocken.in. or thomas.in. or blackman.in. or holder.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:36
-	1312	stichting$.as.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:36
-	200	plasmodium and (kocken.in. or thomas.in. or blackman.in. or holder.in.)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:39
-	7	plasmodium and stichting$.as.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:36
-	2	(plasmodium and (kocken.in. or thomas.in. or blackman.in. or holder.in.)) and "apical membrane antigen"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:39
-	85	AMA-1	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:39
-	4	AMA-1 and (kocken.in. or thomas.in. or blackman.in. or holder.in.)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:40
-	2224	yeast SAME "polyadenylation"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:40
-	117225	protein WITH (expression or production or recombinant)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:41
-	2162	(yeast SAME "polyadenylation") and (protein WITH (expression or production or recombinant))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:41
-	905	((yeast SAME "polyadenylation") and (protein WITH (expression or production or recombinant))) and Pichia	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:41
-	29	(((yeast SAME "polyadenylation") and (protein WITH (expression or production or recombinant))) and Pichia) and plasmodium	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:41

-	27111	glycosylation	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:42
-	22909	glycosylation and (protein WITH (expression or production or recombinant))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:42
-	1376	(glycosylation and (protein WITH (expression or production or recombinant))) and plasmodium	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:42
-	68	((glycosylation and (protein WITH (expression or production or recombinant))) and plasmodium) and (kocken.in. or thomas.in. or blackman.in. or holder.in.)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:42
-	1	(((glycosylation and (protein WITH (expression or production or recombinant))) and plasmodium) and (kocken.in. or thomas.in. or blackman.in. or holder.in.)) and "apical membrane antigen"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:42
-	13	"apical membrane antigen" and glycosylation	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:43
-	88	"apical membrane antigen" or AMA-1	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:43
-	6	("apical membrane antigen" or AMA-1) and (yeast SAME "polyadenylation")	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:44
-	3700	falciparum	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:44
-	31	falciparum and FVO	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:45
-	69	falciparum and ("apical membrane antigen" or AMA-1)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:45
-	59	(falciparum and ("apical membrane antigen" or AMA-1)) and (protein WITH (expression or production or recombinant))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:46
-	53	((falciparum and ("apical membrane antigen" or AMA-1)) and (protein WITH (expression or production or recombinant))) and yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:47
-	2	6066623.pn.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:47
-	0	6066623.pn. and pichia	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:47
-	0	6066623.pn. and yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:47
-	241	ectodomain and plasmodium	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 12:47

-	10	(ectodomain and plasmodium) and "apical membrane antigen"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 13:03
-	925	plasmodium SAME vaccine	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 13:03
-	34299	expression SAME yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 13:04
-	215	(plasmodium SAME vaccine) and (expression SAME yeast)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 13:04
-	19	((plasmodium SAME vaccine) and (expression SAME yeast)) and ("apical membrane antigen" or AMA-1)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 13:09
-	42	(cerevisiae or pastoris) and vaccine and ("apical membrane antigen" or AMA-1)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/09/15 13:10

	Document ID	Title
1	US 20040137512 A1	Malaria plasmodium antigen polypeptide se36, method of purifyng the same and vaccine and diagnostic with the use of the thus obtained antigen
2	US 20030219752 A1	Novel antigen binding molecules for therapeutic, diagnostic, prophylactic, enzymatic, industrial, and agricultural applications, and methods for generating and screening thereof
3	US 20030207287 A1	Non-stochastic generation of genetic vaccines
4	US 6713279 B1	Non-stochastic generation of genetic vaccines and enzymes
5	US 6576757 B1	Polynucleotides encoding flavivirus and alphavirus multivalent antigenic polypeptides
6	US 6479258 B1	Non-stochastic generation of genetic vaccines
7	US 6417341 B1	Malaria merozoite antigen subunit vaccine

	Document ID	Title
8	US 6017734 A	Unique nucleotide and amino acid sequence and uses thereof

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  FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 14:09:05 ON 15 SEP 2004
L1         72025 S PLASMODIUM
L2        250607 S VACCINE
L3          4947 S ECTODOMAIN
L4           515 S "APICAL MEMBRANE ANTIGEN" OR AMA1 OR AMA-1
L5          5989 S KOCKEN?/AU OR HOLDER?/AU
L6         50025 S FALCIPARUM OR (FALCIPARUM (P) FVO)
L7         31760 S GLYCOSYLATION (P) (PROTEIN OR PEPTIDE)
L8        275134 S YEAST OR PASTORIS OR PICHIA
L9           366 S "EXPRESSION OF" (P) L3
L10            7 S L9 AND L8
L11            7 DUP REM L10 (0 DUPLICATES REMOVED)
L12            5 S L11 NOT PY>=2002
L13            1 S L12 AND L1
L14            4 S L9 AND L4
L15            4 DUP REM L14 (0 DUPLICATES REMOVED)
L16          388 S YEAST (S) POLYADENYLATION
L17            0 S L16 AND L4
L18       284093 S "PROTEIN EXPRESSION" OR "RECOMBINANT PROTEIN" OR "PROTEIN PRO
L19            8 S L18 (P) L16
L20            4 DUP REM L19 (4 DUPLICATES REMOVED)
L21            3 S L20 NOT PY>=2002
L22          907 S L7 (P) L18
L23            9 S L22 AND L1
L24            4 DUP REM L23 (5 DUPLICATES REMOVED)
L25            3 S L24 NOT PY>=2002
L26         5599 S L2 AND L1
L27          172 S L26 AND L8
L28          116 S L27 NOT PY>=2002
L29            3 S L28 AND L4
L30            1 DUP REM L29 (2 DUPLICATES REMOVED)
L31           46 S L6 AND L3
L32            1 S L8 AND L31
L33            9 S L18 AND L3 AND L4
L34            7 DUP REM L33 (2 DUPLICATES REMOVED)
L35            2 S L34 NOT PY>=2002
L36            4 S L9 (P) L4
L37            4 DUP REM L36 (0 DUPLICATES REMOVED)
L38           14 S L7 AND L6 AND L8
L39            5 DUP REM L38 (9 DUPLICATES REMOVED)
L40            4 S L39 NOT PY>=2002

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NSWER 4 OF 4 EMBASE
on STN

ACCESSION NUMBER: 97378403 EMBASE
DOCUMENT NUMBER: 1997378403
TITLE: Immunisation with recombinant **AMA-1** protects mice against infection with Plasmodium chabaudi.
AUTHOR: Anders R.F.; Crewther P.E.; Edwards S.; Margetts M.; Matthew M.L.S.M.; Pollock B.; Pye D.
CORPORATE SOURCE: R.F. Anders, Coop. Res. Ctr. Vaccine Technol., Walter/Eliza Hall Inst. Medical Res., Post Office Royal Melbourne Hospital, Melbourne, Vic. 3052, Australia
SOURCE: Vaccine, (1998) 16/2-3 (240-247).
Refs: 22
ISSN: 0264-410X CODEN: VACCDE
PUBLISHER IDENT.: S 0264-410X(97)00178-3
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The Plasmodium merozoite surface antigen **apical membrane antigen**-1 (**AMA-1**) has previously been shown to provide partial protection to Saimiri and rhesus monkeys immunised with recombinant Plasmodium fragile or parasite-derived Plasmodium knowlesi **AMA-1**, respectively. In the study reported here we have used the Plasmodium chabaudi/mouse model system to extend ourpre-clinical assessment of an **AMA-1** vaccine. We describe here the **expression** of the full-length Plasmodium chabaudi adami **AMA-1** and the P. chabaudi adami **AMA-1** **ectodomain** using both baculovirus and Escherichia coli. The **ectodomain** expressed in E. coli which contained an N-terminal hexa-his tag, was purified by Ni-chelate chromatography and refolded in vitro in the presence of oxidised and reduced glutathione to generate intramolecular disulphide bonds. In a series of vaccine trials, in both inbred and outbred mice, highly significant protection was obtained by immunising with the refolded **AMA-1 ectodomain** . Protection was shown to correlate with antibody response and was dependent on intact disulphide bonds. Passive transfer of antibodies raised in rabbits against the refolded **AMA-1** **ectodomain** was also protective. In view of this demonstration that E. coli **expression** of a soluble P. chabaudi **AMA-1** domain can generate a vaccine that is effective in mice, we are pursuing a similar approach to generating a vaccine against P. falciparum for testing in human volunteers.

=>

SWER 1 OF 3 MEDLINE on STN
ACCESSION NUMBER: 1998155832 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9494728
TITLE: Protein expression both in mammalian cell lines and in yeast Pichia pastoris using a single expression plasmid.
AUTHOR: Liu Z; Cashion L M; Pu H
CORPORATE SOURCE: Berlex Biosciences, Richmond, CA 94804-0099, USA.
SOURCE: BioTechniques, (1998 Feb) 24 (2) 266-8, 270-1.
Journal code: 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980422
Last Updated on STN: 19980422
Entered Medline: 19980410

AB We have designed and constructed a novel expression vector capable of producing recombinant proteins in both mammalian cell lines and the yeast strain Pichia pastoris. In this vector, a yeast promoter is placed inside an intron of the mammalian transcription unit. A **yeast** transcription termination sequence is placed immediately downstream of the mammalian **polyadenylation** site. In mammalian cells, transcription is driven by a mammalian promoter. The yeast promoter within the intron is removed by RNA processing. However, **protein expression** in yeast cells can be achieved utilizing the yeast promoter immediately upstream of the 3' splice site and the target genes. Our data indicate that this vector can express beta-galactosidase efficiently in both mammalian cell lines and the yeast strain P. pastoris.

L21 ANSWER 2 OF 3 MEDLINE on STN
ACCESSION NUMBER: 96067159 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7590244
TITLE: The 160-kD subunit of human cleavage-polyadenylation specificity factor coordinates pre-mRNA 3'-end formation.
AUTHOR: Murthy K G; Manley J L
CORPORATE SOURCE: Department of Biological Sciences, Columbia University, New York, New York 10027, USA.
CONTRACT NUMBER: GM 28983 (NIGMS)
SOURCE: Genes & development, (1995 Nov 1) 9 (21) 2672-83.
Journal code: 8711660. ISSN: 0890-9369.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U37012
ENTRY MONTH: 199512
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19980206
Entered Medline: 19951226

AB Cleavage-polyadenylation specificity factor (CPSF) is a multisubunit protein that plays a central role in 3' processing of mammalian pre-mRNAs. CPSF recognizes the AAUAAA signal in the pre-mRNA and interacts with other proteins to facilitate both RNA cleavage and poly(A) synthesis. Here we describe the isolation of cDNAs encoding the largest subunit of CPSF (160K) as well as characterization of the protein product. Antibodies raised against the **recombinant protein** inhibit polyadenylation in vitro, which can be restored by purified CPSF. Extending previous studies, which suggested that 160K contacts the pre-mRNA, we show that purified recombinant 160K can, by itself, bind preferentially to AAUAAA-containing RNAs. While the sequence of 160K reveals similarities to the RNP1 and RNP2 motifs found in many RNA-binding proteins, no clear match to a known RNA-binding domain was found, and RNA

recognition is therefore likely mediated by a highly diverged or novel structure. We also show that 160K binds specifically to both the 77K (suppressor of forked) subunit of the cleavage factor CstF and to poly(A) polymerase (PAP). These results provide explanations for previously observed cooperative interactions between CPSF and CstF, which are responsible for poly(A) site specification, and between CPSF and PAP, which are necessary for synthesis of the poly(A) tail. Also supporting a direct role for 160K in these interactions is the fact that 160K by itself retains partial ability to cooperate with CstF in binding pre-mRNA and, unexpectedly, inhibits PAP activity in in vitro assays. We discuss the significance of these multiple functions and also a possible evolutionary link between **yeast** and mammalian **polyadenylation** suggested by the properties and sequence of 160K.

L21 ANSWER 3 OF 3 EMBASE
on STN

ACCESSION NUMBER: 91138090 EMBASE
DOCUMENT NUMBER: 1991138090
TITLE: Expression of tetanus toxin fragment C in yeast: Gene synthesis is required to eliminate fortuitous polyadenylation sites in AT-rich DNA.
AUTHOR: Romanos M.A.; Makoff A.J.; Fairweather N.F.; Beesley K.M.; Slater D.E.; Rayment F.B.; Payne M.M.; Clare J.J.
CORPORATE SOURCE: Dept. of Molecular Biology, Wellcome Biotech, Langley Court,Beckenham BR3 3BS, United Kingdom
SOURCE: Nucleic Acids Research, (1991) 19/7 (1461-1467).
ISSN: 0305-1048 CODEN: NARHAD
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Fragment C is a non-toxic 50kDa fragment of tetanus toxin which is a candidate subunit vaccine against tetanus. The AT-rich Clostridium tetani DNA encoding fragment C could not be expressed in Saccharomyces cerevisiae due to the presence of several fortuitous **polyadenylation** sites which gave rise to truncated mRNAs. The **polyadenylation** sites were eliminated by chemically synthesising the DNA with increased GC-content (from 29% to 47%). Synthesis of the entire gene (1400 base pairs) was necessary to generate full-length transcripts and for **protein production** in **yeast**. Using a GAL1 promoter vector, fragment C was expressed to 2-3% of soluble cell protein. Fragment C could also be secreted using the α-factor leader peptide as a secretion signal. The protein was present at ~5-10mg/l in the culture medium in two forms: a high molecular mass hyper-glycosylated protein (75-200kDa) and a core-glycosylated protein (65kDa). Intracellular fragment C was as effective in vaccinating mice against tetanus as authentic fragment C. The glycosylated material was inactive, though it was rendered fully active by de-glycosylation.

=>

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on STN

ACCESSION NUMBER: 1999016664 EMBASE

TITLE: High-level expression of **Plasmodium** vivax apical membrane antigen 1 (AMA-1) in **Pichia pastoris**: Strong immunogenicity in Macaca mulatta immunized with P. vivax AMA-1 and adjuvant SBAS2.

AUTHOR: Kocken C.H.M.; Dubbeld M.A.; Van Der Wel A.; Pronk J.T.; Waters A.P.; Langermans J.A.M.; Thomas A.W.

CORPORATE SOURCE: A.W. Thomas, BPRC, Dept. of Parasitology, Lange Kleiweg 157, 2288 GJ Rijswijk, Netherlands. thomas@bprc.nl

SOURCE: Infection and Immunity, (1999) 67/1 (43-49).
Refs: 32
ISSN: 0019-9567 CODEN: INFIBR

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The apical membrane antigen 1 (AMA-1) family is a promising family of malaria blood-stage vaccine candidates that have induced protection in rodent and nonhuman primate models of malaria. Correct conformation of the protein appears to be essential for the induction of parasite-inhibitory responses, and these responses appear to be primarily antibody mediated. Here we describe for the first time high-level secreted **expression** (over 50 mg/liter) of the **Plasmodium** vivax AMA-1 (PV66/AMA-1) **ectodomain** by using the methylotrophic **yeast** **Pichia pastoris**. To prevent nonnative glycosylation, a conservatively mutagenized PV66/AMA-1 gene (PV66Δglyc) lacking N-glycosylation sites was also developed. **Expression** of the PV66Δglyc **ectodomain** yielded similar levels of a homogeneous product that was nonglycosylated and was readily purified by ion-exchange and gel filtration chromatographies. Recombinant PV66Δglyc43-487 was reactive with conformation-dependent monoclonal antibodies. With the SBAS2 adjuvant, **Pichia**-expressed PV66Δglyc43-487 was highly immunogenic in five rhesus monkeys, inducing immunoglobulin G enzyme-linked immunosorbent assay titers in excess of 1:200,000. This group of monkeys had a weak trend showing lower cumulative parasite loads following a **Plasmodium** cynomolgi infection than in the control group.

ANSWER 1 OF 4 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
ACCESSION NUMBER: 2003156537 EMBASE
TITLE: Immunization against Plasmodium chabaudi malaria using combined formulations of **apical membrane antigen**-1 and merozoite surface protein-1.
AUTHOR: Burns Jr. J.M.; Flaherty P.R.; Romero M.M.; Weidanz W.P.
CORPORATE SOURCE: J.M. Burns Jr., Dept. of Microbiology and Immunology, Drexel Univ. College of Medicine, 2900 Queen Lane, Philadelphia, PA 19129, United States. jburns@drexel.edu
SOURCE: Vaccine, (16 May 2003) 21/17-18 (1843-1852).
Refs: 49
ISSN: 0264-410X CODEN: VACCDE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The control of Plasmodium falciparum malaria by vaccination will require immunization with multiple parasite antigens effectively formulated in combination. In this regard, proteins expressed on the surface of blood-stage merozoites are attractive as vaccine targets given their functional importance in the invasion of erythrocytes and accessibility to serum antibodies. We have utilized a Plasmodium chabaudi vaccine model to begin to evaluate the efficacy of immunization with combined formulations of **apical membrane antigen**-1 (**AMA**-**1**) and merozoite surface protein-1 (MSP-1). Using a pET/T7 RNA polymerase bacterial **expression** system, we have expressed, purified and refolded recombinant antigens representing the 54kDa **ectodomain** of Pc **AMA**-**1** and the 42kDa C-terminus of Pc MSP-1. Immunization with recombinant Pc **AMA**-**1**+Pc MSP-1(42) induced a high level of protection against P. chabaudi malaria with protective efficacy varying with antigen dose, choice of adjuvant, and immunization protocol. Based on the reduction of P. chabaudi parasitemia, Alum proved effective for use with the combination of Pc **AMA**-**1** and Pc MSP-1(42). The use of Quil A was similarly effective with single or combined antigen immunizations, particularly with low antigen dose. In general, serological analysis of prechallenge sera indicated a dominant IgG1 response. For a given formulation, immunization with the combination of Pc **AMA**-**1** and Pc MSP-1(42) elicited IgG responses comparable to those observed following immunization with each antigen alone. However, prechallenge antibody titers alone were not predictive of protective efficacy. While Pc **AMA**-**1** and Pc MSP-1(42) can be effectively formulated in combination, further study is needed to define measurable parameters of protective T cell and B cell responses induced by Pc **AMA**-**1**+Pc MSP-1(42) that are predictive of vaccine efficacy.

L15 ANSWER 2 OF 4 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
ACCESSION NUMBER: 2002191222 EMBASE
TITLE: Purification, characterization, and immunogenicity of the refolded ectodomain of the Plasmodium falciparum **apical membrane antigen** 1 expressed in Escherichia coli.
AUTHOR: Dutta S.; Lalitha P.V.; Ware L.A.; Barbosa A.; Moch J.K.; Vassell M.A.; Fileta B.B.; Kitov S.; Kolodny N.; Gray Heppner D.; Haynes J.D.; Lanar D.E.
CORPORATE SOURCE: D.E. Lanar, Department of Immunology, Walter Reed Army Inst. of Research, Forest Glen Annex, Silver Spring, MD

20910, United States. david.lanar@na.amedd.army.mil
SOURCE: Infection and Immunity, (2002) 70/6 (3101-3110).
Refs: 30
ISSN: 0019-9567 CODEN: INFIBR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The **apical membrane antigen** 1 (**AMA1**
) has emerged as a promising vaccine candidate against malaria. Advanced evaluation of its protective efficacy in humans requires the production of highly purified and correctly folded protein. We describe here a process for the **expression**, fermentation, refolding, and purification of the recombinant **ectodomain** of **AMA1** (amino acids 83(Gly) to 531(Glu)) of Plasmodium falciparum (3D7) produced in Escherichia coli. A synthetic gene containing an E. coli codon bias was cloned into a modified pET32 plasmid, and the recombinant protein was produced by using a redox-modified E. coli strain, Origami (DE3). A purification process was developed that included Sarkosyl extraction followed by affinity purification on a Ni-nitrilotriacetic acid column. The recombinant **AMA1** was refolded in the presence of reduced and oxidized glutathione and further purified by using two ion-exchange chromatographic steps. The final product, designated **AMA1**/E, was homogeneous, monomeric, and >99% pure and had low endotoxin content and low host cell contamination. Analysis of **AMA1**/E showed that it had the predicted primary sequence, and tertiary structure analysis confirmed its compact disulfide-bonded nature. Rabbit antibodies made to the protein recognized the native parasite **AMA1** and inhibited the growth of the P. falciparum homologous 3D7 clone in an in vitro assay. Reduction-sensitive epitopes on **AMA1**/E were shown to be necessary for the production of inhibitory anti-**AMA1** antibodies. **AMA1**/E was recognized by a conformation-dependent, growth-inhibitory monoclonal antibody, 4G2dc1. The process described here was successfully scaled up to produce **AMA1**/E protein under GMP conditions, and the product was found to induce highly inhibitory antibodies in rabbits.

L15 ANSWER 3 OF 4 EMBASE
on STN
ACCESSION NUMBER: 1999016664 EMBASE
TITLE: High-level expression of Plasmodium vivax **apical membrane antigen** 1 (**AMA-1**) in Pichia pastoris: Strong immunogenicity in Macaca mulatta immunized with P. vivax **AMA-1** and adjuvant SBAS2.
AUTHOR: Kocken C.H.M.; Dubbeld M.A.; Van Der Wel A.; Pronk J.T.; Waters A.P.; Langermans J.A.M.; Thomas A.W.
CORPORATE SOURCE: A.W. Thomas, BPRC, Dept. of Parasitology, Lange Kleiweg 157, 2288 GJ Rijswijk, Netherlands. thomas@bprc.nl
SOURCE: Infection and Immunity, (1999) 67/1 (43-49).
Refs: 32
ISSN: 0019-9567 CODEN: INFIBR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The **apical membrane antigen** 1 (**AMA**

-1) family is a promising family of malaria blood-stage vaccine candidates that have induced protection in rodent and nonhuman primate models of malaria. Correct conformation of the protein appears to be essential for the induction of parasite-inhibitory responses, and these responses appear to be primarily antibody mediated. Here we describe for the first time high-level secreted **expression** (over 50 mg/liter) of the Plasmodium vivax **AMA-1** (PV66/**AMA-1**) **ectodomain** by using the methylotrophic yeast Pichia pastoris. To prevent nonnative glycosylation, a conservatively mutagenized PV66/**AMA-1** gene (PV66Δglyc) lacking N-glycosylation sites was also developed. **Expression** of the PV66Δglyc **ectodomain** yielded similar levels of a homogeneous product that was nonglycosylated and was readily purified by ion-exchange and gel filtration chromatographies. Recombinant PV66Δglyc43-487 was reactive with conformation-dependent monoclonal antibodies. With the SBAS2 adjuvant, Pichia-expressed PV66Δglyc43-487 was highly immunogenic in five rhesus monkeys, inducing immunoglobulin G enzyme-linked immunosorbent assay titers in excess of 1:200,000. This group of monkeys had a weak trend showing lower cumulative parasite loads following a Plasmodium cynomolgi infection than in the control group.

L15 ANSWER 4 OF 4 EMBASE
on STN

ACCESSION NUMBER: 97378403 EMBASE
DOCUMENT NUMBER: 1997378403
TITLE: Immunisation with recombinant **AMA-1** protects mice against infection with Plasmodium chabaudi.
AUTHOR: Anders R.F.; Crewther P.E.; Edwards S.; Margetts M.; Matthew M.L.S.M.; Pollock B.; Pye D.
CORPORATE SOURCE: R.F. Anders, Coop. Res. Ctr. Vaccine Technol., Walter/Eliza Hall Inst. Medical Res., Post Office Royal Melbourne Hospital, Melbourne, Vic. 3052, Australia
SOURCE: Vaccine, (1998) 16/2-3 (240-247).
Refs: 22
ISSN: 0264-410X CODEN: VACCDE
PUBLISHER IDENT.: S 0264-410X(97)00178-3
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The Plasmodium merozoite surface antigen **apical membrane antigen**-1 (**AMA-1**) has previously been shown to provide partial protection to Saimiri and rhesus monkeys immunised with recombinant Plasmodium fragile or parasite-derived Plasmodium knowlesi **AMA-1**, respectively. In the study reported here we have used the Plasmodium chabaudi/mouse model system to extend ourpre-clinical assessment of an **AMA-1** vaccine. We describe here the **expression** of the full-length Plasmodium chabaudi adami **AMA-1** and the P. chabaudi adami **AMA-1** **ectodomain** using both baculovirus and Escherichia coli. The **ectodomain** expressed in E. coli which contained an N-terminal hexa-his tag, was purified by Ni-chelate chromatography and refolded in vitro in the presence of oxidised and reduced glutathione to generate intramolecular disulphide bonds. In a series of vaccine trials, in both inbred and outbred mice, highly significant protection was obtained by immunising with the refolded **AMA-1** **ectodomain**. Protection was shown to correlate with antibody response and was dependent on intact disulphide bonds. Passive transfer of antibodies raised in rabbits against the refolded **AMA-1** **ectodomain** was also protective. In view of this demonstration that

E. coli **expression** of a soluble P. chabaudi **AMA-1** domain can generate a vaccine that is effective in mice, we are pursuing a similar approach to generating a vaccine against P. falciparum for testing in human volunteers.

SION NUMBER: 96028117 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7588714
TITLE: Molecular and biochemical characterization of a **Plasmodium** falciparum cyclophilin containing a cleavable signal sequence.
AUTHOR: Hirtzlin J; Farber P M; Franklin R M; Bell A
CORPORATE SOURCE: Department of Structural Biology, Biozentrum, University of Basel, Switzerland.
SOURCE: European journal of biochemistry / FEBS, (1995 Sep 15) 232 (3) 765-72.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X85956
ENTRY MONTH: 199512
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19980206
Entered Medline: 19951204

AB The immunosuppressive drug cyclosporin A (CsA) inhibits the growth of malaria parasites in vitro and in vivo. Cyclosporin A exerts its immunosuppressive effect in T lymphocytes by binding to cyclophilin (CyP), a peptidylprolyl cis-trans isomerase (PPIase). It is believed that the cyclosporin/cyclophilin complex inhibits a Ca(2+)-activated **protein** phosphatase, calcineurin, involved in T-cell activation. A cDNA encoding a cyclophilin of the human malaria parasite **Plasmodium** falciparum has been isolated as a step in the elucidation of the mechanism of antimalarial action of CsA. This cDNA, termed PfCyP, encodes a **protein** of 195 amino acids which has highest similarity with the Candida albicans (73.1%) and the Drosophila melanogaster (73.1%) cytoplasmic cyclophilins. A Northern blot reveals an approximately 900-bp nucleotide transcript that is consistent with the predicted size of the encoded polypeptide. The predicted PfCyP **protein** has a putative endoplasmic-reticulum-directed signal sequence at its N-terminus and two potential N-linked **glycosylation** sites. Expression of PfCyP RNA in an in vitro translation/translocation system reveals that the PfCyP **protein** is translocated across microsomes, that the signal **peptide** is cleaved and that the PfCyP **protein** is glycosylated at two sites. The PfCyP cDNA open reading frame coding for the predicted mature **protein** has been expressed in Escherichia coli. The purified **recombinant protein** is an active PPIase (kcat/Km = 2.3 x 10(6) s-1 M-1); this enzymic activity is inhibited by CsA (IC50 = 10 nM). The PfCyP **protein** has thus the same sensitivity to CsA as the PPIase activity associated with P. falciparum extracts [Bell, A. et al. (1994) Biochem. Pharmacol. 48, 495-503] suggesting that PfCyP may be responsible for the PPIase activity in those extracts. If different cyclophilins exist in P. falciparum, we conclude that either the PfCyP **protein** is the major cyclophilin detected in the parasite or that there are other cyclophilins with similar susceptibilities to CsA.

L25 ANSWER 2 OF 3 MEDLINE on STN
ACCESSION NUMBER: 94235214 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7764708
TITLE: Production, purification and immunogenicity of a malaria transmission-blocking vaccine candidate: TBV25H expressed in yeast and purified using nickel-NTA agarose.
AUTHOR: Kaslow D C; Shiloach J
CORPORATE SOURCE: Molecular Vaccine Section, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.
SOURCE: Bio/technology (Nature Publishing Company), (1994 May) 12 (5) 494-9.

Journal code: 8309273. ISSN: 0733-222X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19950809
Last Updated on STN: 20020730
Entered Medline: 19940614

AB We have constructed a second generation malaria transmission-blocking vaccine candidate based on Pfs25, the predominate surface **protein** of **Plasmodium** falciparum zygotes, to overcome potential production problems with the original construct. Four modifications were made: (1) addition of the last cysteine residue of the fourth epidermal growth factor like-domain of Pfs25; (2) mutagenesis of asparagine-linked **glycosylation** sites with glutamine rather than alanine; (3) addition of a six histidine tag at the carboxy-terminus for highly efficient purification of **recombinant protein** on nickel-NTA agarose; and (4) fermentation that combines continuous glucose fed-batch methodology with pH-controlled glucose addition and a terminal ethanol feed. The resulting product, TBV25H (Transmission-Blocking Vaccine based on Pfs25 with a Histidine tag), appears to be a more potent antigen and immunogen than the original construct, and the fermentation and post-fermentation processing methodology easily lend themselves to technology transfer to the ultimate users, newly industrialized countries.

L25 ANSWER 3 OF 3 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2000:227909 BIOSIS
DOCUMENT NUMBER: PREV200000227909
TITLE: Processing and localisation of a GPI-anchored **Plasmodium** falciparum surface protein expressed by the baculovirus system.
AUTHOR(S): Kedees, Mamdouh H.; Gerold, Peter; Azzouz, Nahid; Blaschke, Thomas; Shams-Eldin, Hosam; Muehlberger, Elke; Holder, Anthony A.; Klenk, Hans-Dieter; Schwarz, Ralph T. [Reprint author]; Eckert, Volker
CORPORATE SOURCE: Zentrum fuer Hygiene und Medizinische Mikrobiologie, Philipps-Universitaet Marburg, Robert-Koch-Strasse 17, D-35037, Marburg, Germany
SOURCE: European Journal of Cell Biology, (Jan, 2000) Vol. 79, No. 1, pp. 52-61. print.
CODEN: EJCBDN. ISSN: 0171-9335.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 7 Jun 2000
Last Updated on STN: 5 Jan 2002

AB We describe the expression, in insect cells using the baculovirus system, of two protein fragments derived from the C-terminus of merozoite surface protein 1 (MSP-1) of the human malaria parasite **Plasmodium** falciparum, and their glycosylation and intracellular location. The transport and intracellular localisation of the intact C-terminal MSP-1 fragment, modified by addition of a signal sequence for secretion, was compared with that of a similar control protein in which translation of the GPI-cleavage/attachment site was abolished by insertion of a stop codon into the DNA sequence. Both proteins could only be detected intracellularly, most likely in the endoplasmic reticulum. This lack of transport to the cell surface or beyond, was confirmed for both proteins by immunofluorescence with a specific antibody and characterisation of their N-glycans. The N-glycans had not been processed by enzymes localised in post-endoplasmic reticulum compartments. In contrast to MSP-1, the surface antigen SAG-1 of Toxoplasma gondii was efficiently transported out of the endoplasmic reticulum of insect cells and was located, at least in part, on the cell surface. No GPI-anchor could be

detected for either of the MSP-1 constructs or SAG-1, showing that the difference in transport is a property of the individual proteins and cannot be attributed to the lack of a GPI-anchor. The different intracellular location and post-translational modification of recombinant proteins expressed in insect cells, as compared to the native proteins expressed in parasites, and the possible implications for vaccine development are discussed.

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ANSWER 1 OF 2 EMBASE
on STN

ACCESSION NUMBER: 2001071608 EMBASE

TITLE: Assignment of (1)H, (13)C and (15)N resonances of domain III of the **ectodomain** of **apical membrane antigen** 1 from Plasmodium falciparum [5].

AUTHOR: Nair M.; Hodder A.N.; Hinds M.G.; Anders R.F.; Norton R.S.

CORPORATE SOURCE: R.S. Norton, Biomolecular Research Institute, 343 Royal Parade, Parkville, Vic. 3052, Australia.
ray.norton@bioresi.com.au

SOURCE: Journal of Biomolecular NMR, (2001) 19/1 (85-86).
Refs: 8
ISSN: 0925-2738 CODEN: JBNME

COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Letter

FILE SEGMENT: 004 Microbiology
027 Biophysics, Bioengineering and Medical Instrumentation

LANGUAGE: English